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Retention behaviour of polar compounds using porous graphitic carbon with water-rich mobile phases

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Abstract

The retention factors of polar compounds (mono-, di- and trisubstituted aromatic derivatives) were measured on porous graphitic carbon (PGC), alkyl-modified silicas and an apolar copolymer (PRP-1) with water–methanol mobile phases. It was first shown that the mobile phase effects were similar with the three sorbents and that the comparison of retention factors extrapolated to aqueous mobile phases (k'_w) could give information on stationary phase–solute interactions. The functional group contribution was examined. For aromatic derivatives containing hydrophobic substituents (alkyl and chloro groups), correlations with octanol–water partition coefficients were obtained for the three sorbents. For aromatic derivatives containing polar substituents, these correlations were obtained only for alkylsilicas and PRP-1. On PGC, the retention factor increased with increase in the number of polar substituents and was shown to depend on both the field and the mutual resonance effects of the different substituents on the aromatic ring. The results indicate that electronic interactions are more important than hydrophobic interactions in the retention mechanism of polar compounds. The parametrization of the polarity of the solutes, taking into account field and resonance effects, was carried out using local dipolar moments and the overall electron-excess charge density. The analyte retention factors could be predicted through correlation between log k'_w and the electron-excess charge density.

1. Introduction

One of the objectives in making graphite-based sorbents for use in liquid chromatography (LC) was to provide a reversed-phase stationary phase that would not suffer from the disadvantages of silica-based sorbents, i.e., solubility in the eluent or hydrolysis of the bonded chains at low or high pH and the effects of the unavoidable underivatized silanol groups. After several attempts [1–7], it was only recently that porous graphitic carbons (PGC) were made available, the most common one under the tradename Hypercarb [8]. The PGC sorbent has effectively proved to be unique, showing properties of both reversed-phase (RP) and normal-phase (NP) sorbents, owing to its highly ordered crystalline structure composed of large layers of hexagonally arranged carbon atoms [9–11].

PGC was first described as a stronger reversed-phase sorbent than most C_{18} silicas and provided similar retentions of analytes with a much greater content of organic solvent in the mobile phase. The retention increase caused by the

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addition of a methylene group in a series, considered as a measure of the hydrophobic properties of a RP sorbent, was found to be larger on PGC than on alkyl-bonded silicas [12–14]. Further, the flat rigid surface of PGC allowed unique stereoselectivity of solutes, as illustrated by the separation of geometric isomers and some diastereoisomers, especially under NP conditions [15–18]. Its high stability over the full pH range allowed separations with strongly acidic and basic mobile phases [19,20].

It has also been shown that the retention mechanism was different from that observed on RP silicas [1–5, 21–29]. The delocalization of the π-electrons in the large graphitic bands and the high polarizability of the carbon are responsible for strong dispersion interactions in addition to solvophobic interactions. Tanaka et al. [12] observed that the presence of hydrophilic groups in the molecule did not cause as great a retention decrease on carbon as on C_{18} silica. Postulating a retention mechanism including dispersion forces, they stated that any molecular mass increase in the solute, be it in hydrophobic or dipolar moieties, tends to cause a retention increase. The term "hydrophobic adsorption" was introduced to characterize the positive interaction between the PGC and the solute, as opposed to the "hydrophobic partitioning" observed with C_{18} silicas [14]. Bassler et al. [22] have shown that PGC acted as an electron-pair acceptor of solutes under non-polar conditions. The capacity factors of a set of non-congeneric aromatic solutes with hexane as eluent were related to structural information extracted from nineteen molecular descriptors of solutes by multivariate analysis [21]. The most important for retention were structural features reflecting the abilities of the solutes to participate in intermolecular interactions of electron-pair donor–acceptor and dipole–dipole induced types, whereas structural factors related to molecular size appeared to have little effect on retention.

The compounds considered up to now in most of the studies in the RP mode were non-polar or moderately polar, so that their retention was governed more by hydrophobic interactions than by electronic interactions. In previous studies, we have shown the high affinity of PGC towards very polar and water-soluble compounds in water-rich mobile phases [27–29]. For example, the capacity factor of 1,3,5-trihydroxybenzene was about 1000 in water as mobile phase whereas this compound was not retained at all by C_{18} silica and was even proposed as an experimental probe to measure the void volume of C_{18} columns. This high retention of polar compounds added a new future to PGC, i.e., a potential for extracting very polar or water-soluble contaminants that could not be extracted by any method up to now from environmental waters and that are consequently very difficult to determine at trace levels.

The objective of the work presented here was to acquire a better knowledge of the retention behaviour of polar analytes with PGC in order to collect more information about the retention mechanism and to be able to predict which compounds possess high retention factors in water. Data were collected for aromatic derivatives containing one or more polar groups. The capacity factors were also measured using two other reversed-phase sorbents, i.e., C_{18} silica and the apolar styrene–divinylbenzene copolymer PRP-1, for which the retention mechanism was known. Comparison of data obtained with the three sorbents allowed a better understanding of the respective effects of the polar and the electronic character of the interactions involved in the retention mechanism with PGC.

2. Experimental

2.1. Apparatus

Retention measurements were carried out with a Model 5060 liquid chromatograph equipped with a UV 200 variable-wavelength spectrophotometer (Varian, Palo Alto, CA, USA) or an electrochemical detector (Coulochem Model 5100; ESA, Bedford, MA, USA.).

2.2. Stationary phases and columns

Commercially available columns (100×4.6 mm I.D.) packed with Hypercarb porous graphitic carbon of 7-μm particle size (Shandon,

Runcorn, UK), a laboratory-packed column (100 × 4.6 mm I.D.) containing PRP-1 copolymer of 10-μm particle size (Hamilton, Reno, NV, USA), laboratory-packed columns (100 × 4.6 mm I.D.) containing LiChrosorb RP-8 or RP-18 of 5-μm particle size (Merck, Darmstadt, Germany) or a 250 × 4.6 mm I.D. column prepacked with the spherical 5-μm octylsilica Zorbax (Interchim, Paris, France) were used for retention measurements. Laboratory-made stainless-steel precolumns (22 mm × 4.6 mm I.D. or 27 mm × 4.6 mm I.D.) were used for some retention measurements in water-rich mobile phases. They were laboratory-packed using a thick slurry and a microspatula. The void volume was determined for each mobile phase by injection of 2 *M* sodium nitrate solution.

2.3. Chemicals

HPLC-grade acetonitrile was obtained from Rathburn (Walkerburn, UK) and methanol from Prolabo (Paris, France). LC-grade water was prepared by purifying demineralized water in a Milli-Q filtration system (Millipore, Bedford, MA, USA). Other chemicals were obtained from Prolabo, Merck or Fluka (Buchs, Switzerland). Solutions of selected solutes were prepared by weighing and dissolving them in methanol.

2.4. Calculations of electron-excess charge density

The charge distribution was calculated using the SYBYL molecular modelling package (version 6.0) running on a Vax 3200/VMS (version 5.42). The molecular mechanics force field implemented in the SYBYL program produced molecular geometries close to crystal structures. A model was calculated for each molecule. The different conformations were systematically examined and the lowest energy conformation was selected by taking into account its atomic charge distribution. The charge distribution was calculated by the semi-empirical molecular orbital method MNDO provided in the MOPAC5 package.

3. Results and discussion

Retention factors were measured for the set of mono-, di- and trisubstituted aromatic derivatives reported in Table 1. In order to compare data obtained with the three reversed-phase sorbents, the role of the mobile phase was first investigated.

3.1. Comparison of mobile phase effects

In chromatography, the retention factor can be generally defined as the ratio of the sum of the interactions between the solute and the mobile phase and the sum of the interactions between the solute and the stationary phase. Therefore, comparison of retention factors can give information on interactions with the sorbents provided that the sum of interactions between the solutes and the mobile phase is expected to be similar with the three sorbents and provided that the phase ratios are similar. We do not intend to compare the absolute retention values for one sorbent with another, but some relationships with solute structures or their retention order or the effect of some groups, so that a knowledge of the phase ratio is not required. However, comparison at a given composition of the mobile phase was impossible, owing to the polarity range of the compounds of interest, because some of them were not retained and others were too retained for measurements. Therefore, it was decided to measure the retention factors in mobile phases made of water and methanol over the largest range possible and to extrapolate the data to pure water conditions. In water-rich mobile phases, short analytical columns were laboratory packed to obtain shorter retention volumes.

Fig. 1 shows the variations of the experimental retention factors of three analytes, benzene, phenol and acetophenone, with the methanol volume fraction of the mobile phase for C_{18} silica, PRP-1 and PGC. These three analytes were selected because the shapes of the curves were different. The variations were linear in a certain range of methanol concentrations and deviation from linearity was observed for benzene and for acetophenone with mobile phases

Table 1
Extrapolated retention factor values ($\log k'_w$) from the linear variations $\log k'$ versus volume fraction of methanol in the mobile phase for each benzene derivative for the three stationary phases, C_{18} silica, PRP-1 and PGC. The range of methanol used for the measurements is indicated in square brackets. Compounds are characterized by their water–octanol partition coefficient ($\log P_{oct}$). The positions of the substituents in the benzene ring are indicated by the number in brackets.

Solute: functional group			Log P_{oct}	Extrapolated $\log k'_w$				
				C_{18}	PRP-1		PGC	
Monosubstituted								
$CH(CH_3)_2$			3.68	–	5.55	[80, 100]	3.5	[50, 80]
C_3H_7			3.55	–	5.35	[80, 100]	3.4	[50, 80]
C_2H_5			3.15	3.2	4.85	[70, 90]	2.85	[30, 60]
CH_3			2.74	2.75	4.2	[60, 80]	2.3	[20, 70]
H			2.14	2.25	3.5	[0, 60]	1.5	[0, 70]
Cl			2.84	2.8	nd	–	2.65	[20, 90]
OCH_3			2.08	2.15	nd	–	2.2	[20, 80]
COOH			1.86	1.9	3.3	[40, 80]	2.45	[30, 90]
NO_2			1.84	2.05	3.6	[60, 90]	2.45	[50, 100]
$COCH_3$			1.70	2.0	2.9	[0, 80]	2.5	[20, 90]
CN			1.67	1.8	nd	–	2.1	[30, 80]
OH			1.50	1.55	2.4	[0,90]	1.8	[0, 100]
CHO			1.5	1.55	2.9	[50, 80]	1.8	[20, 50]
CH_2OH			1.10	1.5	2.45	[0, 60]	2.2	[30, 50]
NH_2			0.9	1.10	2.5	[20, 80]	1.35	[0, 60]
Disubstituted								
(1) OH	(2) OH		1.0	nd[a]	1.6	[0, 90]	2.0	[0, 100]
(1) OH	(3) OH		0.8	0.5	1.25	[0, 70]	2.3	[0, 100]
(1) OH	(4) OH		0.6	nd	0.85	[0, 70]	2.20	[0, 100]
(1) COOH	(2) OH			nd	3.05	[30, 70]	2.8	[50, 90]
(1) COOH	(3) OH		2.4	nd	2.4	[30, 70]	2.6	[50, 70]
(1) COOH	(4) OH		2.3	nd	2.3	[30, 70]	2.65	[50, 90]
(1) NH_2	(2) NH_2		0.15	nd	1.7	[10, 60]	1.4	0, 60]
(1) NH_2	(3) NH_2			nd	1.35	[0, 60]	1.6	[0, 60]
(1) NH_2	(4) NH_2			nd	1.2	[0,50]	2.3	[0, 60]
(1) OH	(2) NH_2		0.52	nd	1.7	[30, 60]	1.7	[20, 90]
(1) OH	(3) NH_2		0.17	nd	1.3	[30, 60]	1.6	[20, 90]
(1) OH	(4) NH_2		0.04	nd	1.1	[30, 60]	2.05	[20, 90]
(1) COOH	(2) NH_2		1.21	nd	2.85	[30, 60]	2.9	[30, 60]
(1) COOH	(3) NH_2			nd	1.6	[30, 60]	2.8	[30, 60]
(1) COOH	(4) NH_2		0.68	nd	2.0	[30, 60]	2.85	[30, 60]
(1) NO_2	(2) NO_2		1.58	1.9	3.95	[70, 90]	2.7	[70, 100]
(1) NO_2	(3) NO_2		1.49	1.8	4.0	[70, 90]	3.6	[80, 100]
(1) NO_2	(4) NO_2		1.46	1.7	4.05	[70, 90]	3.1	[80, 100]
(1) COOH	(2) COOH		0.79	nd	2.2	[30, 50]	2.75	[40, 80]
(1) COOH	(3) COOH		1.67	nd	3.1	[30, 60]	3.5	[40, 80]
(1) NH_2	(4) NO_2		1.39	1.5	2.85	[30, 60]	3.0	[60, 90]
(1) OH	(4) NO_2		1.91	2.0	2.7	[30, 60]	3.1	[70, 100]
Trisubstituted								
(1) OH	(2) OH	(3) OH		nd	0.8	[0, 70]	2.2	[0, 100]
(1) OH	(3) OH	(5) OH		nd	0.5	[0, 70]	2.7	[0, 100]
(1) COOH	(2) OH	(3) OH		nd	2.4	[30, 70]	2.9	[50, 90]
(1) COOH	(2) OH	(6) OH		nd	2.85	[30, 70]	2.65	[50, 90]
(1) COOH	(3) OH	(4) OH		nd	1.5	[30, 70]	2.95	[50, 90]
(1) COOH	(3) OH	(5) OH		nd	1.35	[30, 60]	3.0	[50, 90]
(1) COOH	(2) COOH	(3) COOH		nd	1.95	[30, 50]	3.95	[50, 80]
(1) COOH	(3) COOH	(5) COOH	1.15	nd	2.6	[30, 50]	4.5	[70, 90]
(1) OH	(2) Cl	(4) NH_2		nd	2.85	[30, 60]	2.45	[30, 90]

[a] nd = Not determined.

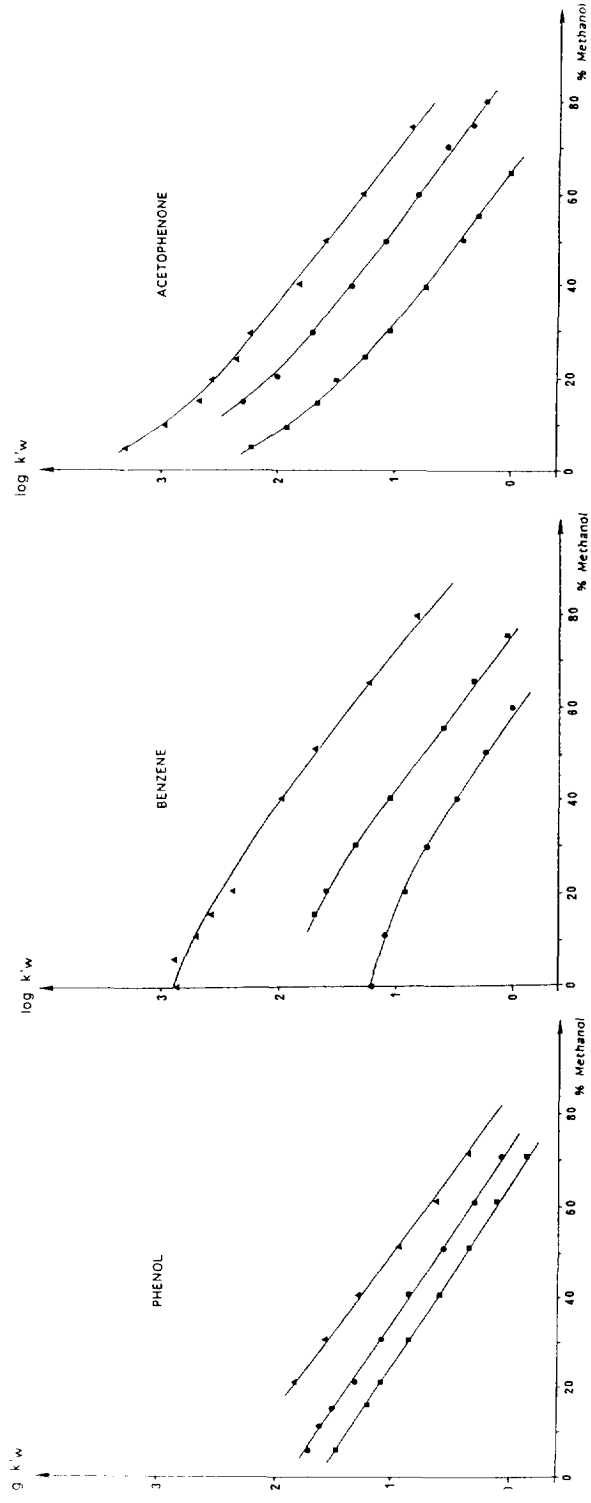

Fig. 1. Variations of the logarithm of the retention factor ($\log k'$) with the volume fraction of methanol in the mobile phase for benzene, phenol and acetophenone. ■ = RP-18 silica; ▲ = PRP-1; ● = PGC.

containing more than 75% water. These variations are well known for alkylsilicas and have been the subject of many studies. As pointed out by Schoenmakers et al. [30], for some analytes, the linear variation turned out to be quadratic when the whole range of methanol content was investigated. The retention factor in water, k'_w, was extrapolated from the linear log k'–methanol volume fraction relationship and the difference between the extrapolated and the real values can be positive or negative depending on the solutes. In a previous study, we had shown that the more polar the analyte, the less retained it was by C_{18} silicas and the closer the extrapolated and experimental values were [31].

However, the most important point to be considered in the comparison of experimental variations obtained for each stationary phase is the parallelism of the curves. The same deviations from the linear range are observed for the three sorbents, which are striking results. With C_{18} silicas, the retention process has received much attention but the fundamental aspects are still unclear as to whether the retention is described by a partition or an adsorption process, or a combination of the two. However, it is well established that there is preferential wetting of the alkyl chains by the mobile phase organic solvent which causes an imprecise demarkation of the mobile and stationary phases, that the conformation of the chains can change with variations in the experimental conditions and finally that the retention of some molecules can occur by an NP process on surface silanol groups as well as by an RP process. To a first approximation, at low water concentrations, it was shown that water is adsorbed to a slight extent and at higher water concentrations starting at ca. 10–30% (v/v), the organic component is adsorbed [32]. We have demonstrated that in a limited range of composition from around 20% to 70% of water, the change in retention was mainly due to the change in the solubility of the solute in the mobile phase [33]. In this range, when only the methanol content of the mobile phase varies in the chromatographic system, the variation of the retention factor results only from the variation of the interactions between the mobile phase and the solute, the interactions between the stationary phase and the solute being constant in this range. This classical view of the passive role of the bonded ligand was recently challenged and some experiments have shown that the free energy of retention can arise also from net attractive processes in the stationary phase [34].

It is therefore difficult to explain the parallelism of the curves observed in Fig. 1, except that the same preferential wetting is observed with change in composition of methanol. To a first approximation, the results indicate that since the same interactions between the solutes and the mobile phase are observed with the three sorbents, the interactions between the stationary phase and the solute are of course different from one sorbent to another, but for each sorbent they are constant in the limited range of mobile phase composition where the relationship is linear. Therefore, for a given composition of the mobile phase in the linear range, the difference in retention factor from one sorbent to the other is related to the difference in interactions with the stationary phases and can be measured by the nearly constant difference in log k' between the curves. Comparison of data extrapolated to pure water from the linear range can be a first approach for comparison of sorbent–solute interactions.

The extrapolated values of log k'_w are reported in Table 1, together with the water–octanol partition coefficients. The experimental range of mobile phase composition giving a linear relationship is also reported. The retention factors of di- and trisubstituted derivatives were not determined on C_{18} silicas because, in general, they are not retained by this sorbent. As some compounds are ionizable, the pH of the mobile phase was adjusted in order that the analyte should be in its molecular form.

3.2. Comparison of log k'_w data

In the literature, PGC is often compared with C_{18} silicas and is described as a stronger hydrophobic sorbent. The results reported in Table 1 for monosubstituted derivatives indicate that the log k'_w values are higher on C_{18} than on PGC for ethylbenzene, toluene, chlorobenzene and ben-

zene and that the reverse order is observed for a polar functional group on substituted aromatic rings. This comparison does not allow one to compare hydrophobic properties and the methylene increment, $\alpha(CH_2)$, that can be roughly estimated from our data for the alkyl derivatives is higher for PGC (average $\log \alpha = 0.55$) than for C_{18} silica (average $\log \alpha = 0.45$). These results are consistent with more accurate values of $\alpha(CH_2)$ calculated from measurements of the retention times of alkanols and reported to be 4.50 and 3.84, respectively [14]. No comparison was made with apolar copolymers, but PRP-1 shows higher values for each monosubstituted compound. The differences are fairly high, the retention of benzene being 100 times higher on PRP-1 than on PGC. The methylene increment is found to be slightly higher for PRP-1 (average $\log \alpha = 0.60$) than for PGC.

The results are very different for di- and trisubstituted analytes. Most of the analytes contain polar functional substituents and are characterized by $\log P_{oct.}$ values below 1.5. All these compounds were weakly retained by C_{18} silica and their $\log k'_w$ values were not evaluated. For PRP-1, a close examination of the data indicates that the retention factor of a compound having two polar aromatic substituents is always lower than that measured for each corresponding monosubstituted benzene, whereas the opposite is observed with PGC. Except for dinitro derivatives, the $\log k'_w$ values are higher on PGC than on PRP-1. The difference is especially large for very polar compounds such as some dihydroxy-, diamino- or hydroxyamino-substituted and other trisubstituted derivatives.

As the hydrophilic properties of analytes decreases with increasing number of substituted polar groups, these results indicate first a different behaviour of PGC from that of C_{18} silica or PRP-1 copolymer.

3.3. Correlation with octanol–water partition coefficients ($P_{oct.}$)

The relationship between the hydrophobic parameter $\log P_{oct.}$ and the extrapolated $\log k'_w$ from methanol–water measurements obtained with C_{18} silicas is well known, although it can only be an approximation, as pointed out by Kaliszan [35] in a recent review of quantitative structure–retention relationships (QSRR) applied to reversed-phase chromatography. Even if extrapolated $\log k'_w$ values have no physical meaning, it is an easy means of normalizing retention, provided that appropriate C_{18} silicas are used and that extrapolated data are derived from water–methanol measurements [36]. Braumann [36] gathered many extrapolated $\log k'_w$ data obtained with these conditions and good relationships between $\log k'_w$ values and $\log P_{oct.}$ were observed for a large number of compounds differing in polarity and chemical properties. These correlations suggest that retention with C_{18} silicas is primarily governed by hydrophobic effects, which closely reflect the water–octanol partition coefficients [35,36]. These correlations were examined with our experimental data on the three sorbents (see below).

Octadecylsilicas

The relationship between the $\log k'_w$ data in Table 1 and $\log P_{oct.}$ is shown in Fig. 2a. The slope of 0.92 ± 0.03 is in agreement with other published work. The more hydrophilic the analyte is, the lower its retention is and the correlation in Fig. 2a leads to an approximate value of $\log k'_w$ with no measurement.

Apolar copolymer PRP-1

The same correlation is also represented in Fig. 2a for the styrene–divinylbenzene porous copolymer PRP-1. Values of $\log k'_w$ were determined for a large number of compounds with this sorbent, although trisubstituted derivatives were not represented here because their $\log P_{oct.}$ values were not found. A perfect linear relationship is observed for the series of alkylbenzenes. For other compounds, the correlation is not really linear but a general trend is visible and indicates that $\log k'_w$ decreases with increase in $\log P_{oct.}$, showing that the retention is also mainly governed by hydrophobic interactions. Strong electron–donor interactions can be involved, especially π–π interactions with the aromatic ring of the solutes due to aromatic rings in the network of the polymer matrix, in addition to

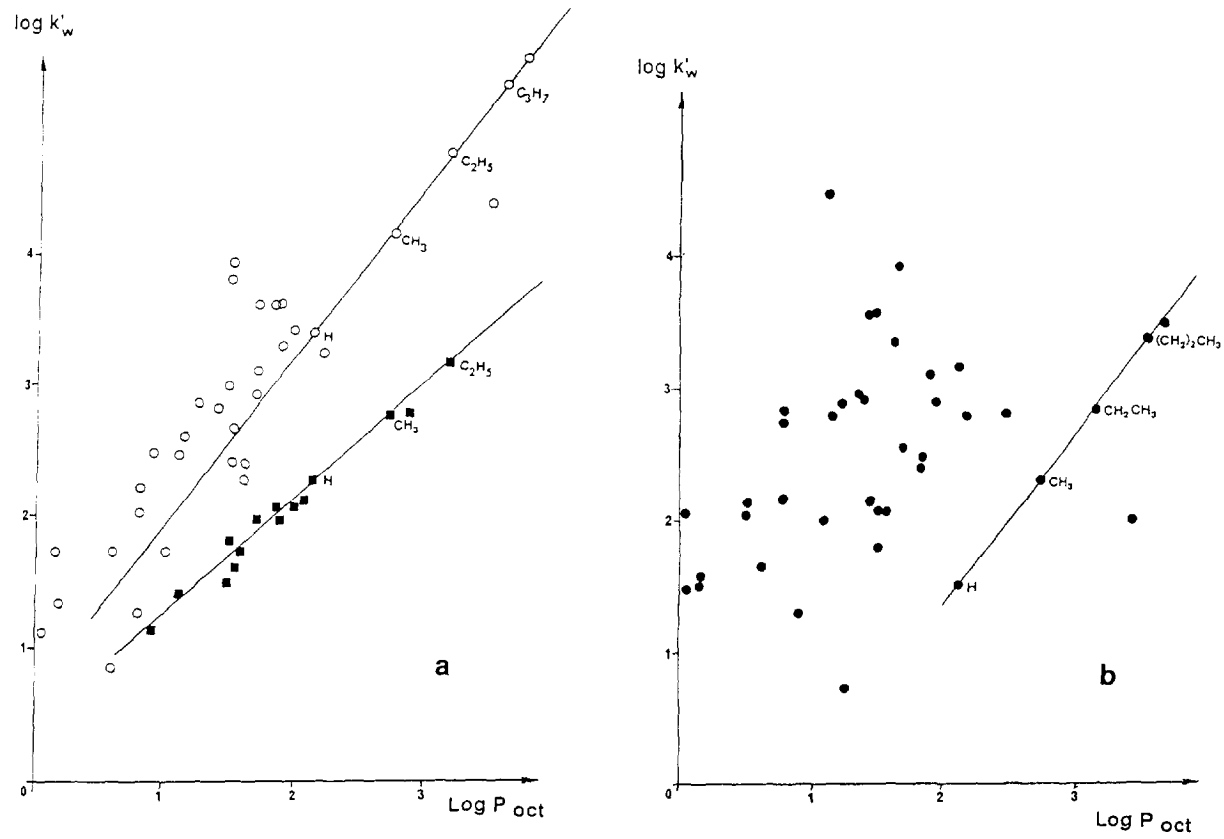

Fig. 2. Relationship between $\log k'_w$ and $\log P_{oct.}$ (a) $\log k'_w$ values obtained on (■) C_{18} silica and (○) PRP-1 copolymer; (b) $\log k'_w$ values on PGC. See Table 1 for solutes.

hydrophobic interactions. Therefore, the interaction is sensitive to changes in the electron density of the solute caused by the electron-withdrawing or -donating ability of the substituent. The correlation between $\log k'_w$ and $\log P_{oct.}$ cannot be similar to that obtained with C_{18} silicas, because some substituents can have both a hydrophobic and an electronic effect. As PRP-1 can only be a π-donor, the effect of strong electron-withdrawing substituents such as nitro groups is responsible for a higher retention, and the $\log k'_w$ values of these compounds are effectively above those corresponding to alkylbenzenes. On the other hand, the $\log k'_w$ values of compounds having strong electron-donating substituents such as amino or hydroxy groups are lower. We previously compared the retention data of moderately polar compounds characterized with $\log P_{oct.}$ values above 1.5 and found that the k'_w data for PRP-1 were about 25–50 times higher than those for C_{18} silicas [28]. Obviously, the lower $\log P_{oct.}$ is, the smaller is the difference in $\log k'_w$ and very polar analytes are only slightly more retained by PRP-1 than they were by C_{18} silicas. The limitation of using PRP-1 for extracting polar degradation products of atrazine from water has already been pointed out for compounds with $\log P_{oct.}$ values below 1 [37].

Porous graphitic carbon

As can be seen in Fig. 2b, there is no trend between solute hydrophobicity and retention for the set of compounds examined in this study. Hydrophobic interactions are not the most important interactions that govern the retention

mechanism. If more compounds containing only hydrophobic moieties were to be studied, the trend should be obtained. This is illustrated by the linear relationship observed for the series of alkylbenzenes, which includes chlorobenzene.

3.4. Functional group contribution

Monosubstituted

The functional group contribution is usually determined by the difference (τ_X) in the logarithm of retention factors of the two analytes which differ by the presence and absence of the functional group X of interest. The functional group contribution to RP liquid chromatography was recently reviewed by Smith [38]. In Table 2, the τ_X values calculated from extrapolated $\log k'_w$ values in pure water are reported for the three sorbents. The groups have been classified in order of decreasing τ_X values on C_{18} silica and the same order is observed for the characterization of the groups by their Hansch π constants. Since this parameter takes mainly into account both the size and the polarity of the functional group, the relationship (not presented here) between τ_X and the Hansch constant is similar to that obtained between $\log k'_w$ and $\log P_{oct.}$ and in agreement with the hydrophobic retention mechanism. The Hansch π value is useful for characterizing the "polarity" of groups. Polar groups are often characterized by negative Hansch values, whereas groups with positive values are often named "hydrophobic groups".

Little work has been devoted to the retention behaviour of analytes with polystyrene–divinylbenzene copolymers. The capacity factors of some mono- and disubstituted aromatic compounds were compared with those of C_{18} silicas and it was found that the contributions for the hydrophobic groups were more positive and for the more polar groups more negative [39,40]. In Table 2, the τ_X values on PRP-1 shows about the same decreasing order as the Hansch π constant, except for nitro and hydroxy groups. As stated, these results can be explained by the electronic effects of the group on the benzene ring. The Hammett equation allows one to characterize the different functional groups by a σ value that sums the resonance and field effects of a group X when attached to a benzene ring. This value correlated many reaction data, especially for groups in *meta* and *para* positions [41]. The σ values are reported in Table 2, a positive value indicating an electron-withdrawing group and a negative value an electron-donating group. The high σ value for the nitro group explains the difference in retention on PRP-1. However, the close values of τ_X for hydroxy and amino groups are more difficult to explain.

The results for PGC are different and no

Table 2
Functional group contribution for various aromatic compounds

Substituent X	τ_X values on sorbent			Hansch constant	Hammett parameter
	RP-18	PRP-1	PGC		
Cl	0.5	–	1.11	0.71	0.23
CH_3	0.46	0.74	0.80	0.56	–
$COOCH_3$	−0.08	–	1.66	−0.01	0.44
OCH_3	−0.15	–	0.68	−0.02	−0.28
COOH	−0.30	−0.1	0.92	–	0.44
NO_2	−0.25	0.22	0.78	−0.28	0.80
$COCH_3$	−0.30	−0.30	1.03	−0.55	0.47
CN	−0.57	–	0.54	−0.57	0.70
OH	−0.80	−1.0	0.28	−0.67	−0.38
NH_2	−1.20	−0.92	−0.20	−1.23	−0.57

The values of τ_X are defined as $\log k'_{RX} - \log k'_{RH}$, R being the aromatic ring and X the substituent. Values of k' extrapolated to pure water according to Table 1.

correlation can be found with the σ constant, even for the nitro group.

Polysubstituted

The information given for polysubstituted derivatives is more interesting. For C_{18} silica and PRP-1, generally, we can predict if the retention will increase or decrease on adding a second group to the benzene ring, depending on its Hansch π constant value: whatever the first group attached on the aromatic ring, any addition of a hydrophobic group will cause an increase in retention, whereas any addition of a polar group will cause a decrease.

On PGC, an increase in retention is always observed when a second group is added, as shown from the comparison of values in Table 1. Fig. 3 shows the increase in $\log k'_w$ data with the number of OH, or COOH groups in the aromatic molecules. A linear relationship is obtained for monosubstituted, disubstituted in positions 1 and 3 and trisubstituted in positions 1, 3 and 5. This increase in retention with the increasing number of NO_2 groups in similar positions has been already pointed out by Colin et al. [42], working with experimental pyrocarbon-modified silicas. However, when considering the different positions and when adding a second nitro group in position 2 or 4, the retention increases, but not as much as for position 3. This will be explained below by the different magnitudes of the resonance effects in the different positions on the ring.

These results indicate that the interactions between PGC and solutes are mainly dispersive (London) interactions. Because of the high polarizability of the π-electrons, especially strong dispersion interactions can occur with molecules possessing conjugated π-electrons. This explains why the highest retention is obtained for substituents in a position where the resonance effect between the polar functional groups and the benzene ring is at a maximum.

A specific feature of London interactions is their additivity. Therefore, the energy of interaction is approximately the sum of the individual energies between each group. Golkiewicz et al. [7] have shown that the increment of $\log k'$ on

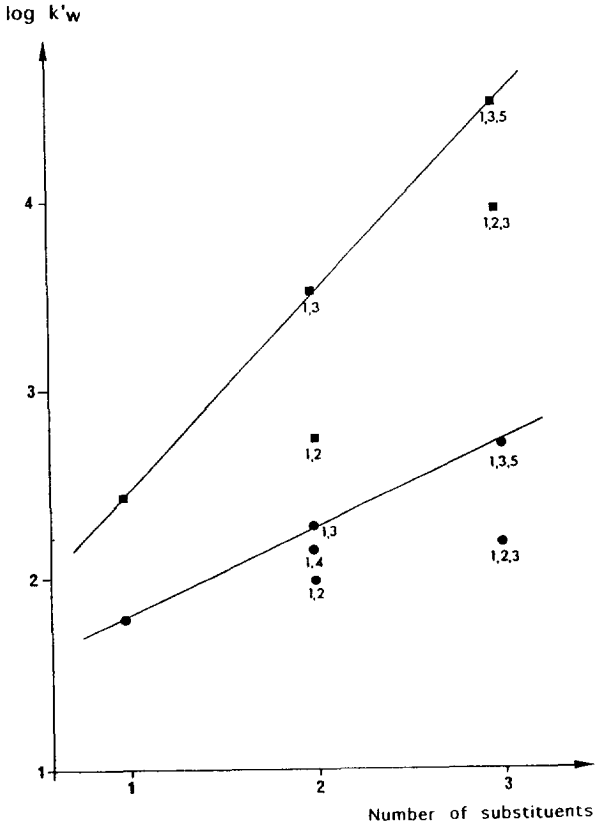

Fig. 3. Variation of $\log k'_w$ with the number N of (●) hydroxy or (■) carboxylic groups on the aromatic ring. The numbers indicate the positions of the substituents on the ring.

adding a CH_3 or a Cl group in an aromatic molecule was constant and did not depend on the nature of the first substituent or on the position of the added group. However, CH_3 and Cl groups are hydrophobic and do not induce resonance effects. Table 3 shows the increment in $\log k'_w$ observed on adding an OH group in position 2, 3 or 4 to a benzene ring already substituted by a Y group. Low increments were observed when the first group was hydrophobic, Cl or CH_3, whereas increments between 0.16 and 0.72 were obtained with polar Y groups. It can be seen that the increment depends on the position of the added OH group. Again, the resonance effect induced by the OH group depends on that induced by the first substituent and on the respective positions of the two

Table 3
Increment in $\log k'_w$ for polar monosubstituted aromatic derivatives (Y being the substituent) when a hydroxy group is added to the ring at position 2, 3 or 4

Substituent Y	OH in position 2	OH in position 3	OH in position 4
NO_2	–	–	0.72
COOH	0.36	0.16	0.20
NH_2	−0.24	0.24	0.63
OH	0.22	0.40	0.25
CH_3	−0.06	−0.06	–
Cl	0.02	0.12	0.07
OCH_3	–	–	0.40
$COCH_3$	–	0.24	–
$COOCH_3$	–	–	0.18

substituents. Our results indicate that both the polarity of the functional groups and the "induced" polarity on the ring by the different substituents have to be taken into account for predicting retention on PGC. More investigation is required, taking into account also steric or intramolecular effects of substituents and mobile phase interactions.

3.5. Polarity parametrization of solutes

Dispersive interactions are of the instantaneous dipole-induced dipole type and can occur between electrically neutral molecules that do not possess any permanent dipole. Kaliszan [35] classified the structural descriptors of solutes used in QSRR analysis into physico-chemical parameters and others related to bulkiness, to geometry or shape, to polarity and to topology. Dipole moments, atomic excess charges, orbital energies, superdelocalizabilities and partially charged surfaces can describe polarity-related or electronic parameters. In QSSRs describing RP-LC retention on C_{18} silicas, various descriptors more or less directly linked to molecular size were not able to account at the same time for structurally specific polar differences, so a polarity parameter was added [43]. It was shown that the overall dipole performed poorly, but it is well known that some symmetrical molecules (e.g., a benzene ring with two identical polar groups in positions 1 and 4) can have an overall dipolar moment near zero and behave as polar solutes. Kaliszan [44] therefore introduced a submolecular measure of the polarity reflecting the largest molecular local dipole in the molecule. A solute in specific contact with a stationary phase has at least one of its fragments close to the interacting surface and, therefore, the polar interaction of a solute would logically be better described by local molecular dipoles. This quantum chemical polarity parameter, Δ, was calculated by the largest difference in the individual atomic electronic excess charges in the molecule. This calculation required first the determination of the electron densities on all the atoms and then locating the atom with the highest electron excess and the atom with the highest electron deficiency.

3.6. Interactions with PGC

Tables 4 and 5 report the local dipolar moments corresponding to the various substituents for nitro- and dinitrobenzenes and for phenol and some di- and trihydroxybenzenes. The overall positive electron-excess charge density of the benzene ring in the molecule and the overall negative electron-excess charge density of the whole molecule, including the polar substituents, have also been reported. Nitro- and dinitrobenzene are known to be strong π-acceptors, since both the electron-withdrawing (or resonance) and the electronic field effects are negative. The benzene ring bears a positive charge [Σq_+(ring)] and the nitro group a negative charge. The

Table 4
Values of $\log k'_w$ on RP-18, PRP-1 and PGC for nitro- and dinitrobenzenes, submolecular polarity parameter Δ, overall positive electronic charge of the benzene ring, Σq_+(ring), and overall negative excess-electron charge, Σq_-

Parameter	Nitro-	1,2-Dinitro-	1,3-Dinitro-	1,4-Dinitro-
$\log k'_w$ (RP-18)	2.05	1.9	1.8	1.7
$\log k'_w$ (PRP-1)	3.6	3.95	4.0	4.05
$\log k'_w$ (PGC)	2.45	2.7	3.6	3.1
Δ_1	0.67	0.53	0.69	0.60
Δ_2		0.53	0.68	0.60
Σq_+(ring)	0.19	0.32	0.74	0.52
Σq_-	−1.08	−1.04	−1.79	−1.50

Δ is the difference in the electronic charges borne by the N atom of the nitro group and the adjacent C atom in the ring.

highest local dipolar moment was found between the N atom of the nitro group and the C atom of the ring. Owing to the different locations of the resonance effects, both the Δ_1 and Δ_2 values for 1,2- and 1,4-dinitrobenzene are lower than the Δ value for nitrobenzene, whereas higher values are observed for 1,3-dinitrobenzene. The position of the second nitro group also has a different effect on the increase of the positive ring charge. As shown in Table 4, the retention order on PGC agrees with the sum of the highest local dipolar moments, with the positive charge of the ring and also with the overall negative electron-excess charge density. On C_{18} silica, the retention decreases on adding a second nitro group, whereas on PRP-1, this is an example where the retention increases with increase in the number of substituents, owing to the $\pi-\pi$ interactions between the π-electron-donor character of PRP-1 and the strong positive electron-excess charge density of the ring of the dinitrobenzenes.

The same data are reported for hydroxybenzenes in Table 5. The field effect of a hydroxy group is negative whereas the resonance effect is positive. Evidently, owing to the much lower positive electron-excess charge density of the ring, no correlation is found between the retention order and the ring charge, so that the interaction cannot be explained in term of $\pi-\pi$ interactions, as on PRP-1. The position on the ring of a second or of a third OH group strongly influences the values of Δ and each of them is the highest where it is in positions 1, 3 and 5. A correlation is observed between the sum of the Δ values and the retention order, indicating that all the polar groups interact at the same time with the PGC surface and should be taken into account in the total interaction. As the solute molecule is planar, the interactions occur be-

Table 5
Values of $\log k'_w$ on PGC for phenol and polyhydroxybenzenes, submolecular polarity parameters Δ, overall positive electronic charge of the benzene ring, Σq_+(ring) and overall negative electron-excess charge, Σq

Parameter	Phenol	1,2-Dihydroxy	1,3-Dihydroxy	1,4-Dihydroxy	1,2,3-Trihydroxy	1,3,5-Trihydroxy
$\log k'_w$	1.8	2.0	2.30	2.15	2.20	2.7
Δ_1	0.34	0.35	0.38	0.32	0.30	0.41
Δ_2		0.27	0.40	0.32	0.34	0.44
Δ_3		–	–	–	0.32	0.43
Σq_+(ring)	0.90	0.16	0.13	0.13	0.20	0.18
Σq_-	−0.58	−0.74	−0.94	−0.79	−0.98	−1.32

Δ is the difference in the electronic charges borne by the oxygen atom and the carbon atom adjacent to it in the ring.

tween the local dipoles and the flat, rigid surface of the layers in the PGC structure, the dispersion interactions thus being favoured in the perpendicular direction to the PGC layers. Kaliszan [44] pointed out that a special form of dispersion interactions can be observed in large molecules containing π-electrons such as the PGC layers which can simply be described as gigantic aromatic molecules composed entirely of carbon. In these molecules, the length of the fluctuating instantaneous dipole can be so great that the actual interactions are of individual charges changing their position and not the instantaneous dipoles. This form of dispersion interaction is in agreement with the fact that all the polar substituents account for retention, the PGC adapting its fluctuating charges to the different local dipoles in the molecule. Depending on the position of the substituents, local dipoles are not equivalent, as shown by the calculation from the electron density on particular atoms, which is an effective means of quantifying the inter-substituent effect. The local dipolar moments were sometimes difficult to calculate for polar groups such as COOH and some others. The overall negative electron-excess charge density, Σq_-, was therefore calculated and we found that the highest resonance effect was always characterized by the highest Σq_- value, as shown for 1,3-dinitrobenzene or 1,3-dihydroxy- and 1,3,5-trihydroxybenzene. A high Σq_- value corresponds also to stronger and/or more numerous local dipoles. Fig. 4 shows that there is good agreement between Σq_- and $\log k'_w$ obtained for phenol and di- and trihydroxybenzenes; the deviation observed for 1,2,3-trihydroxybenzene is certainly due to steric hindrance of the three groups.

The overall negative electron-excess charge density was calculated for all the molecules in Table 1. Fig. 5 shows the variation of $\log k'_w$ with the corresponding Σq_- values. We can observe a trend showing the importance of the overall negative electron-excess charge density for the retention. The more heteroatoms are present in the molecule, the more resonance effects are observed between the ring and the substituents and the higher the retention of the analyte is.

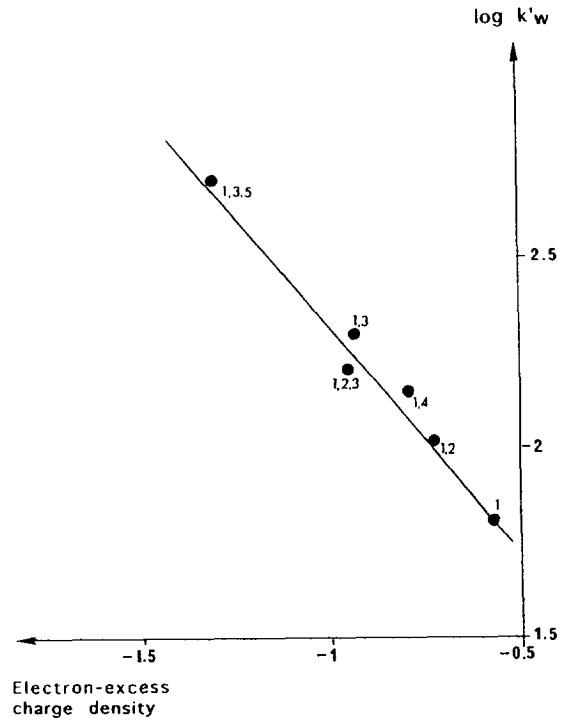

Fig. 4. Relationship between $\log k'_w$ and the overall negative electron-excess charge density, Σq_-, of phenol and hydroxybenzenes. The numbers indicate the positions of the hydroxy groups on the benzene ring.

The value of Σq_- is a means of quantifying the electronic characteristics, i.e., the electronic charges of the heteroatom and the induced resonance effects. The correlation in Fig. 5 is similar to that observed between $\log k'_w$ and $\log P_{oct.}$ with C_{18} silicas. It is obtained over a wide variation in $\log k'_w$ from 1 to 4.5 and can be used for obtaining an approximate value of retention. On the basis of instantaneous dipole (or individual charges)-induced dipole interactions, the correlation should be better with the sum of the square of local dipolar moments, taking into account the polarizability of the medium. However, this correlation requires some more complicated calculations.

This result was obtained for very polar compounds. Regarding alkylbenzenes, characterized by similar Σq_- values, the $\log k'_w$ data are situated on a vertical line and do not fit the curves, the retention of these compounds being

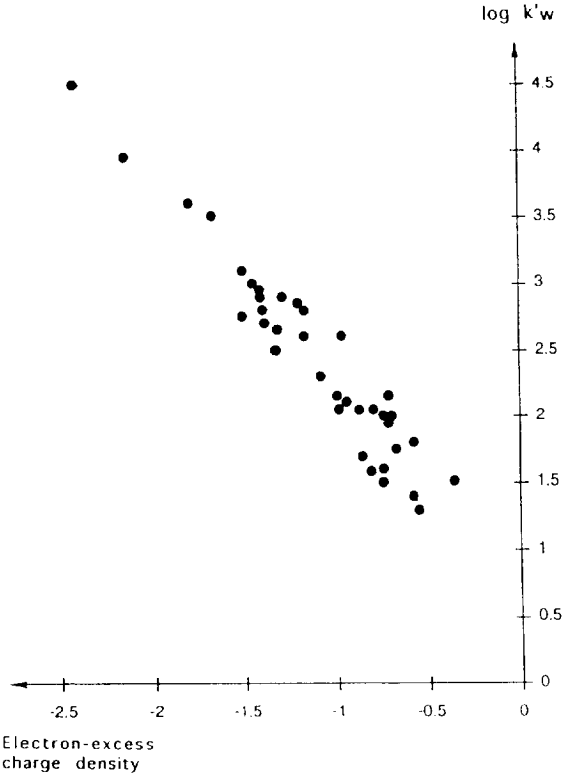

Fig. 5. Relationship between $\log k'_w$ and the overall negative electron-excess charge density, Σq^-, of benzene derivatives substituted by polar groups. See Table 1 for compounds.

mainly due to both hydrophobic and small dispersion interactions that are not additive. Comparison between the respective contributions of hydrophobic and electronic interactions would require more data for apolar and moderately polar compounds.

4. Conclusion

The porous graphitic carbon retains polar compounds fairly well under reversed-phase conditions. It is obvious now that the retention behaviour of solutes is different from that observed with other reversed-phase sorbents. The prediction of the retention factors of analytes requires a good knowledge of the interactions involved in the retention process. The work presented here is a small contribution, showing that electronic interactions are more important than hydrophobic interactions in the retention mechanism of polar compounds. More data should be collected to widen our knowledge of the PGC behaviour. A better knowledge of the parameters that would allow the characterization of the polarity of molecules is also necessary.

The high retention of some very polar compounds in water confirms the unique potential of PGC as an extraction sorbent for trace analyses for some water-soluble micropollutants.

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